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TIME-RESOLVED ANALYTICAL PYROLYSIS MASS SPECTROMETRY OF BIOMATERIALS

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This report documents method development for time-resolved analytical pyrolysis applied to microorganisms and biomaterials. A direct insertion probe (DIP) was the biological sample processor and was inserted into an electron ionization source of a quadrupole mass spectrometer (MS). E. coli, B. subtilis and various biochemical compounds were investigated for biomarker information content. The DIP-MS approach provides an additional dimension toward solving the difficult problems of generation and trace descriptor fragments from complex biomaterials.

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PREFACE

The work described in this report was authorized under Project No. 1C162622A553C, Reconnaissance, Detection and Identification. This work was started in March 1990 and completed in October 1990.

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TIME-RESOLVED ANALYTICAL PYROLYSIS MASS SPECTROMETRY OF BIOMATERIALS

1. INTRODUCTION

Analytical pyrolysis of biopolymers and microorganisms has benefited from the pioneering research of Reiner (1960's), Morgan (1970's) and Meuzelaar (1970's) (1). Taxonomy of bacteria was made possible by correlations of fingerprint portions primarily to the cell wall lipopolysaccharides and lipoproteins (2a). Further development of the fatty acid products analysis involved extraction/derivatization for capillary GC (2b) determinations for both species and genus classification. Forensic science and food industry applications developed following the pyrolysis fragmentation/chromatography research that provided informative "pyrograms." Pyrolysis mass spectrometry (Py-MS) and pyrolysis GC-MS applications are now extended from the recent reports by Voorhees et al (3) and Snyder et al (4). Voorhees et al. showed that Curie point pyrolysis-MS could provide fatty acid fragments from whole microorganisms that were informative for identification purposes. Snyder et al. demonstrated advantages of including a gas chromatography (GC) separation of the pyrolyzate prior to the ion trap detector analysis of the underivatized fatty acid fragments.

This report documents method development for time-resolved analytical pyrolysis applied to microorganisms and biomaterials. The approach provides an additional dimension toward solving the difficult problems of generation and detection of trace descriptor fragments from complex materials. Programmed or time-resolved analytical pyrolysis was demonstrated in 1978 (5) to provide unique information related to synthetic polymer microstructure and degradation mechanisms. Since that time, wide-ranging applications were conducted throughout the industrial R&D community with the commercially available systems.

Precise thermal control and versatile interfacing configurations to spectral detectors (such as Fourier transform infrared and MS units) were used with the Pyroprobe systems for detailed microstructural information (6). The present configuration permits both pulse (millisecond risetime up to a final temperature of 1400°C) or slow, programmed treatments (from 5°C/min. to 300°C/min.) over time intervals from 1 to 240 minutes. In the present work, exploitation of the controlled thermal system was made with on-line analysis by direct interfacing to a Finnigan TSQ MS/MS system. In this manner, rather than relying on extensive data analysis to provide "a chemometric" separation, programmed pyrolysis provided raw data that were amenable to a straightforward, visual analysis. The raw data were found to be informative in a microbiological sense with respect to the microorganism

analyte. Thermal programming or time-resolved pyrolysis emphasizes low activation energy fragmentation/reaction processes. Pulse or rapid risetimes to a final set temperature emphasize high activation energy processes. The pyrolysis mode is common for polymer microstructural "fingerprinting." Oftentimes, however, selective bond-breaking with programmed pyrolysis may provide details in the fragmentation process to augment the pulse pyrolysis information.

The combination of a thermal separation with on-line monitoring by MS and, with potential extended method development to MS/MS analysis, provides new means with which to study bio- and synthetic materials. A thermal-MS degradation profile is demonstrated in this exploratory series for selected samples, including E. coli, B. subtilis, polyglycine, a tripeptide (glycylglycylglycine) and the famous Murchinson meteorite with unknown organic contents. The time-resolved pyrolysis method is evaluated for effects of the programmed rate to provide discriminating information using three data comparisons: (1) conventional mass spectral patterns from individual or summed scansets (mass spectral sequences in time) (2) semi-quantitative data from total or reconstructed (selected) ion chromatograms (TIC or RIC) (3) three-dimensional mass spectral mapping, identified as Pyrolysis Mass Maps (PyMM).

2. EXPERIMENTATION

A Pyroprobe Model 122 was used to control a modified direct insertion Pyroprobe (DIP) unit. The DIP was interfaced to a Finnigan TSQ-MS/MS Model 4500 as shown schematically in Figure 1. As with all DIP units, interfacing to various spectral units requires careful attention to engineering design tolerances, control and calibration considerations. A ramp output of the programmed pyrolysis was recorded for the selected rates of 60°C, 120°C, and 300°C/min, as well as 1000°C, 1200°C, and 1300°C pulse treatments. Figure 2 shows minimal overshoot and exact ramp reproducibility throughout the control ranges of the DIP unit for 40°, 60°, and 120°C/min. ramps to 1000°C. Modification to the standard control/calibration circuitry was required for the smaller Pt filament with the Finnigan TSQ. The design permits sample pyrolyzate to be delivered directly into the ion volume. The 20-30 microgram sample is contained in a quartz tube closed at one end that is inserted into the Pt filament coil in the standard manner.

The TSQ was used in the electron ionization (EI) operating mode and was easily converted to and from its standard use as a pyrolysis/concentrator GC-MS system. Mass spectral data (m/z 30-600) were collected at the rate of 1 scan per second. Software provided with the INCOS data system was used for the three selected data formats to evaluate the effect of time-resolved pyrolysis on the biomaterial thermal fragmentation.

3. RESULTS AND DISCUSSION

3.1 General.

The effectiveness of time-resolved analytical pyrolysis for the study of microorganisms is considered in three formats:

3.1.1 Conventional qualitative identification by mass spectral patterns obtained from individual scans or summed scansets (selected timeframes) with library search comparisons:

3.1.1.1 RICs of selected scanset(s) showing the mass spectral pattern in the full m/z 30-600 range.

3 1.1.2 Library search results comparing sample mass spectra to the three best matches in the National Institute of Science and Technology mass spectral library.

3.1.2 Semi-quantitative evaluation of the RIC of selected ions in the scanset(s). The ratio of the ion counts of selected mass spectral regions to the TIC ion counts was used to calculate the percentage of pyrolyzate with respect to the indicated ions.

3.1.3 Pyrolyzate intensity/time/mass distribution patterns are identified as Pyrolysis Mass Maps (PyMM).

3.2 Experimental Data for E. Coli.

3.2.1 Slow (40°C/min.) Programmed Pyrolysis to a Final Temperature of 1000°C.

3.2.1.1 A relatively slow programming of 40°C/min was conducted to the initial fragmentation region of about 400°C. The ramp was manually switched to 20°C/min. between about 400-500°C, then resumed at 40°C/min. to the final 1000°C set temperature. Figure 3(a-c) shows the selected m/z ranges and the scanset maximum compared to the TIC with a maximum intensity at scanset #1274. Figure 3(b) shows that low amounts of the desired high molecular weight fragments (m/z 299, 400-600) were formed by examination of scansets #1240-1250 and #1240-1270. The maximum production of these ions occurs at scanset #1249, notably earlier than the TIC maximum. Figure 4(a) presents the mass spectral pattern for m/z 30-550 contained in scansets #1240-1250 and Figure 4(b), for m/z 250-600 in that same time frame. These data agree with the mass spectral pattern obtained from earlier work by Snyder, et al. using the pyrolysis GC/ion trap detector system which showed maximum peaks at m/z 299 and 522 for E. coli (4). A typical library search is shown in Figure 4(c). Figure 4(d) shows selected, averaged mass spectra at various times over the E. coli TIC. Note that as the TIC is interrogated over the pyrolysis envelope, a

greater amount of noise is introduced and the higher molecular weight material are observed.

3.2.1.2 Semi-quantitative assessment of the m/z fragments denoted above are given in Table 1. Selected scansets containing the three regions of interest (m/z 30-299, 300-600, and 400-600) are presented as a percentage of the ion counts (RIC) to the total ion counts (TIC). Most of the fragmentation (91%) is accounted for by m/z 30-299 fragments in a timeframe of 3 minutes, maximizing at #1273 scanset. The high m/z species (300-600) in scansets #1267, 1299, and 1280 account for ca. 0.9% of the species (4). The earliest informative fragments from the E. coli run were observed in scansets #1230-1250 (between 1:30-1:50 min.) and account for ca. 0.4% (589) counts of the TIC. The highest series, m/z 400-600, are ca. 0.35% of the pyrolyzate.

Table 1

Selected, Averaged Scanset(s) at 40°C/min.

m/z	Scansets	% of Pyrolyzate*
30-299	#1240-1250	91
300-600	#1267, 1299	0.6
400-600	#1230-1250	0.35

*Ratioed with respect to the TIC (-100%).

3.2.1.3 The PyMM in Figure 5 presents the intensity/time/mass for the m/z 50-299 (x1) and 299-600 (x20) regions. The PyMM permits a comparison of the relative pyrolyzate fragment distribution (m/z 50-600) over the full timeframe of the programmed pyrolysis run. The predominant m/z 299 envelope is noted, as well as a lower amount of the m/z 522 species evolving in the same time interval.

3.2.2 Moderate (120°C/min.) Programmed Pyrolysis to a Final Set Temperature of 1000°C.

3.2.2.1 Figure 6 presents the selected RIC and TIC, indicating their respective intensity maxima, for m/z 30-299 (Fig. 6(a)); m/z 299 and 400-600 (Fig. 6(b)); and m/z 400-600 (Fig. 6(c)). The mass spectral patterns are presented from scansets #220-240 for m/z 50-300 (Fig. 7(a)) and for m/z 300-600 (Figure 7(b)). The predominant series (differing by a $-CH_2$ unit) with a strong m/z 522 ion was observed. Figure 7(c) portrays selected, averaged mass spectra at various times over the 120°C/min. E.

coli TIC. Similar conclusions can be drawn with that of the 40°C/min. TIC (Figure 4(d)). Figure 8(a) and (b) records typical library search results.

3.2.2.2 Table 2 presents the semi-quantitative results from the E. coli sample for a comparison to that seen in Table 1 programmed at the slower (40°C/min.) rate. The m/z 30-299 region accounted for a similar amount (92%) of the total fragmentation as did the slower ramped run (91%), but the m/z 300-600 fragments were notably higher (1.5%), with m/z 400-600 recorded at 0.25% of the total pyrolyzate (similar to 0.35% in Table 1). The combined m/z 299, 400-600 accounted for ~0.3%, since m/z 299 alone in scansets #230-250 was only 0.06%. The m/z 299 ion was the earliest detected "significant" species (488 counts) in scansets #230-250).

Table 2

Selected, Averaged Scansets at 120°C/min.

m/z	RIC scansets	% of Pyrolyzate*
30-299	#220-240	92%
300-600	#220-240	1.5%
400-600	#210-260	0.25%
299	#230-250	0.06%

*Ratioed with respect to the TIC (=100%).

3.2.2.3 The PyMM of E. coli results from the time resolved 120°C/min. run is shown in Figure 9. A sharper production of m/z 299 species is noted, and a better yield in the higher molecular weight fragments is recorded. Visual comparison of Figure 9 to Figure 5 clearly shows an improved focus of the product distribution to more informative fragments. It may be concluded for the E. coli experiment that slow heating through the sensitive degradation stages (20°C/min in scansets #1220-1240) notably alters fragmentation pathways. To improve generation of the higher molecular weight ions, a faster (120°C/min.) programmed pyrolysis was shown to be effective. The PyMM data format provided visual comparisons that focused on the relative amounts of the m/z 299 and 522 regions and an improved distribution of fragments in the 300-600°C temperature region.

3.3 Experimental Results for B. Subtilis.

Programmed pyrolysis at 60°C/min. to a final set temperature of 1000°C produced the results seen in Figures 10-12. Importantly, Figure 11(b) shows the production of fragments with an m/z 550 maximum, in

agreement with the pattern noted in reference (4). Table 3 presents the selected RIC (scansets) for the m/z ranges of interest ratioed to the TIC. As with E. coli, m/z 30-299 accounts for the predominant portion of fragmentation (95%, vs. 92% for E. coli). The important m/z 300-600 region scanset (#445-465) gave 0.5% of the total pyrolyzate (vs. 1.5% for E. coli), but 0.63% for m/z 299 vs. 0.06% for E. coli. Figure 11(c) presents the selected, averaged mass spectra of the B. subtilis pyrolysis TIC. Note the increase in noise and decrease in the intensities of the informative high molecular weight lipid components as increasing temperatures are investigated.

Table 3

Selected, Averaged Scansets at 60°C/min.

m/z	RIC scansets	% of Pyrolyzate
30-299	#453 max	95.1
50-600	#430-470	61.7
300-600	#445-465	0.5
299	#454 max	0.63

*Ratioed with respect to the TIC (~100%).

The PyMM (Figure 12) indicates a similar focus on product distributions in the higher mass regions (m/z 299-600), as did the 120°C/min. run for E. coli. The distinctively different overall pattern for B. subtilis in the m/z 450-550 region is clearly seen in the expanded (x20) portion of the PyMM. Also, the absence of fragments in the m/z 300-450 region should be noted, contrary to the extensive fragment distribution seen in the PyMM of E. coli.

3.4 Experimental Results for Polyglycine.

Programmed pyrolysis at 60°C/min. to a final set temperature of 1000°C resulted in the data presented in Figures 13-15. These pyrolysis conditions give a typical protein fragmentation distribution to be used for comparison to the more complex microorganism samples. More than 99% of the pyrolyzate is due to fragment ions m/z 30-299. Predominant fragments typical of proteins appear at m/z 44, 56/57, 68/69, 84/85, 114, 125, 157 and 165 (Figure 14(a)) and a typical library search for scansets #455-475 is shown in Figure 14(b). The PyMM for m/z 50-299 is useful as a reference "group pattern" perhaps to compare with the m/z 30-299 portion of complex protein-containing samples, such as the microorganisms (Figures 5, 9 and 12).

3.5 Experimental Results for the Glycylglycylglycine Tripeptide.

Programmed pyrolysis at 60°C/min. for the tripeptide to a set final temperature of 1000°C gave the results presented in Figure 16-18. Pyrolyzate fragments in the m/z 30-299 region gave a maximum at scanset #430 (Figure 16(a)). The mass spectral pattern for scans #420-430 is shown in Figure 16(b), with a library search (Figure 17) giving a fair match for the dipeptide, n-glycylglycine. As with polyglycine, over 99% of the pyrolyzate is accounted for in the m/z 30-299 region. The PyMM of 50-299 is sharply focused compared to the more complex protein (Figure 15). Key fragments of m/z 44, 56, 71, 76, and 114 are predominant from the tripeptide.

3.6 Experimental Results for Murchinson Meteorite, Water-Soluble Portion.

Programmed pyrolysis at 60°C/min. to a set final temperature of 1000°C resulted in the data seen in Figures 19-22. The TIC is shown for the pyrolyzates along with the m/z 50-299 (Figure 19(a)) and m/z 300-500 (Figure 19(b)) RICs. The latter fragments have a maximum late in the time-resolved pyrolysis, (scanset #594). The important mass spectral pattern for scansets #590-610 in the m/z range of 220-550 is shown in Figure 20 as well as scansets #610-630, for m/z 150-600. The ions at m/z 265/267, 365, 393, 407/408, 421, 435, 449, 463, 477 are important evidence of high molecular weight species present in the water soluble portion from the meteorite. The decrease in the TIC intensity in the time-resolved run is shown in more detail in the #610-630 scansets (Figure 20(c)). The relative amounts for the high molecular fragments (m/z 299-600 in scansets #450-650) is ca. 0.4%, a similar low level to that seen from the time-resolved pyrolysis for B. subtilis (0.5%) and E. coli (1.6%). The lower molecular weight series, m/z 50-299, is ~35% of the TIC pyrolyzate, and is well below that seen for the reference protein, polyglycine (>99%), or the tripeptide (>99%). A more complete picture of the mass spectral patterns throughout the TIC is seen in Figure 21, with the time/scan frames shown between #560-570, #570-590, #590-610 and #620-660. It appears that the two series of peaks centered at m/z 265 and 407 are generated in the same timeframe and in the same relative ratios to the total pyrolyzate under these experimental conditions.

The PyMM shows the distribution between m/z 50-299 (x 0.3) and m/z 299-600 (x20) at 60°C/min time-resolved ramp. The broad distribution throughout the 50-299 m/z range might be expected, since many studies given in the literature have documented amino acids, hydrocarbons, and complex, low to medium molecular weight species in this meteorite (7). However, no reports have been cited that show higher molecular weight species such as that observed in these DIP-TSQ time-resolved runs.

A repeat run was conducted a month later with similar experimental conditions on another sample taken from the water-soluble

portion. Similar results were observed with respect to Figures 19-22. In light of the time-resolved pyrolysis results from the microorganisms and reference biopolymer/tripeptide materials, these results are important to the scientific community in the cosmochemistry and origins-of-life fields, as well as to NASA space research programs.

4. CONCLUSIONS

Programmed pyrolysis at slow to moderate rates (20°, 40°, 60° and 120°C/min) to a 1000°C final set temperature provide useful new microstructural information for biologicals, such as microorganisms and biopolymers/tripeptides. Product distributions were shown to be altered depending upon the selected thermal treatment which could provide a means to emphasize the generation of relatively high molecular weight, information-rich fragments. Data treatments such as the RIC and TIC information gave semi-quantitative data of the product distribution, with selected scansets providing specific mass spectral patterns that were compared with library search data.

Importantly, a visual means to compare complex time-resolved analytical pyrolysis results used resident TSQ software in the form of mass spectral maps. The PyMM gave insights to direct the programmed pyrolysis towards the most informative fragmentation pathways, within limits of the experimental setup. The ability to pyrolyze biomaterials through selective fragmentation regimes to enhance lipid-related species has been demonstrated in this study. The results with E. coli and B. subtilis are directly analogous with those obtained in Snyder et al (4) using pulsed pyrolysis, short column GC/ion trap MS equipment. Also, results from time-resolved pyrolysis/GC-ion trap MS at selected rates of 60, 120, 180, and 300°C/min showed potential for the time-resolved pyrolysis method (7). It is evident that advanced pyrolysis instrumentation interfaced properly to MS-based detectors can provide significant capabilities for trace detection and identification/classification of biomaterials.

5. FUTURE RESEARCH

Real-time monitoring of pyrolysis fragments by MS provides advantages compared to more lengthy, restricted time-frame analysis with on-line GC separations. Time-resolved pyrolysis permits analysis of complex thermal product distributions and allows some control of focusing on the desired higher molecular weight range. Future studies are needed to build upon this approach, including extended combinations of thermal treatments to delineate sensitive fragmentation pathways for biomaterials. Importantly, the thermally-induced fragmentations are physically separated from the complex ion-induced mechanisms that occur in other MS-based configurations (i.e., fast ion bombardment (FAB), SIMS, etc.)

The sample-limited nature of DIP-MS analyses is a problem for practical applications: e.g., real-time mass spectral pattern evaluations

from pyrolysis of complex environmental samples. Studies of selected thermal separation programs (time-resolved pyrolyses that optimize the desired information) should thus include an extended sample processing aspect; i.e., thermal treatments with concentrator/GC or MS capabilities. This method workup would be analogous to the dynamic headspace/pyrolysis programmed thermal treatments used with bulk formulated polymeric products. The automated sequencing of interface/pyrolyzer provides enhanced selectivity in the analysis.

Furthermore, nonthermal sample processing, such as supercritical fluid (SF) technology for complex materials, has shown successful results in separation of lipid-related species (8). Combination of SF extraction (SFE) with direct interfacing to MS units would provide the needed combination of diverse sample handling with specific spectral data. Prototype interfaces, such as TRANSCAP, are being developed for such time-dependent, difficult analyses requiring large sample amounts (mg to gm) (9). This approach would handle trace organic analyses of low amounts of target species in bulk matrices with potential real-time MS or Fourier Transform Infrared (FTIR) detection/identification.

Finally, the DIP-MS TSQ results have led to development of a specialty DIP unit for trace amounts of particulates/aerosols in air analyses. Design of an Aerosol DIP for interfacing to the TSQ will be based on the information gained from the present work. A potential means to detect and identify organics/microorganisms sampled from air has been indicated by the Aerosol DIP concept. Instrumentation and software to quantitate and to classify these data could be extended for MS and FTIR systems using advanced sample processing capabilities discussed above.

Also, extension of MS capabilities of the TSQ system would include method development for MS/MS analyses of the time-resolved pyrolysis mode. In this application, however, scansets would likely include a multitude of fragments, rather than a discrete parent ion. The mass filter would, therefore, use a "mass window" in a selected scanset(s) region and serve as a means to amplify low level regions of the mass maps, i.e., PyMM obtained at high sensitivity in the MS/MS mode for ultratrace detection of target masses.

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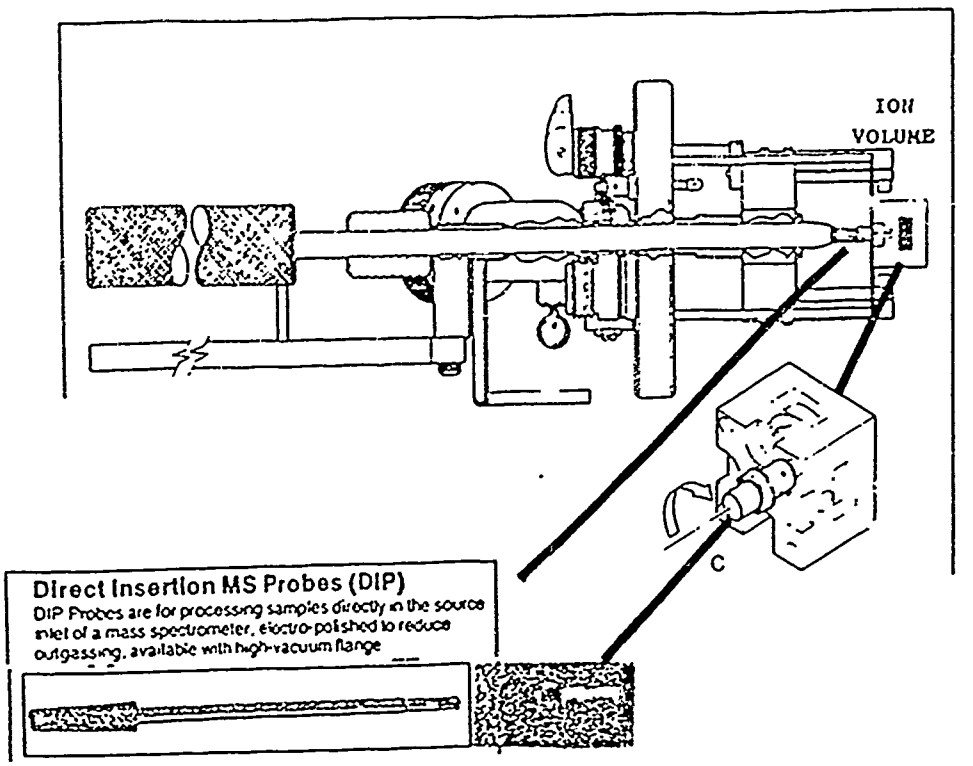
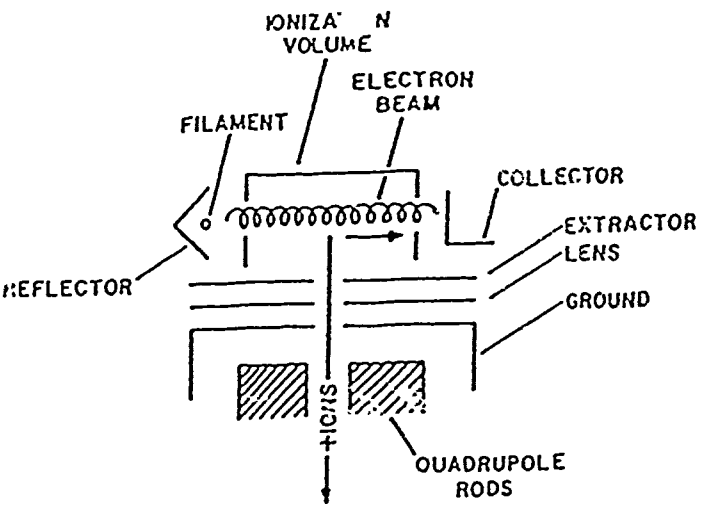




Electron Impact Ion Source.

Figure 1. Pinnigan TSQ MS/MS Direct Insertion Pyroprobe, DIP-MS, Model 4500

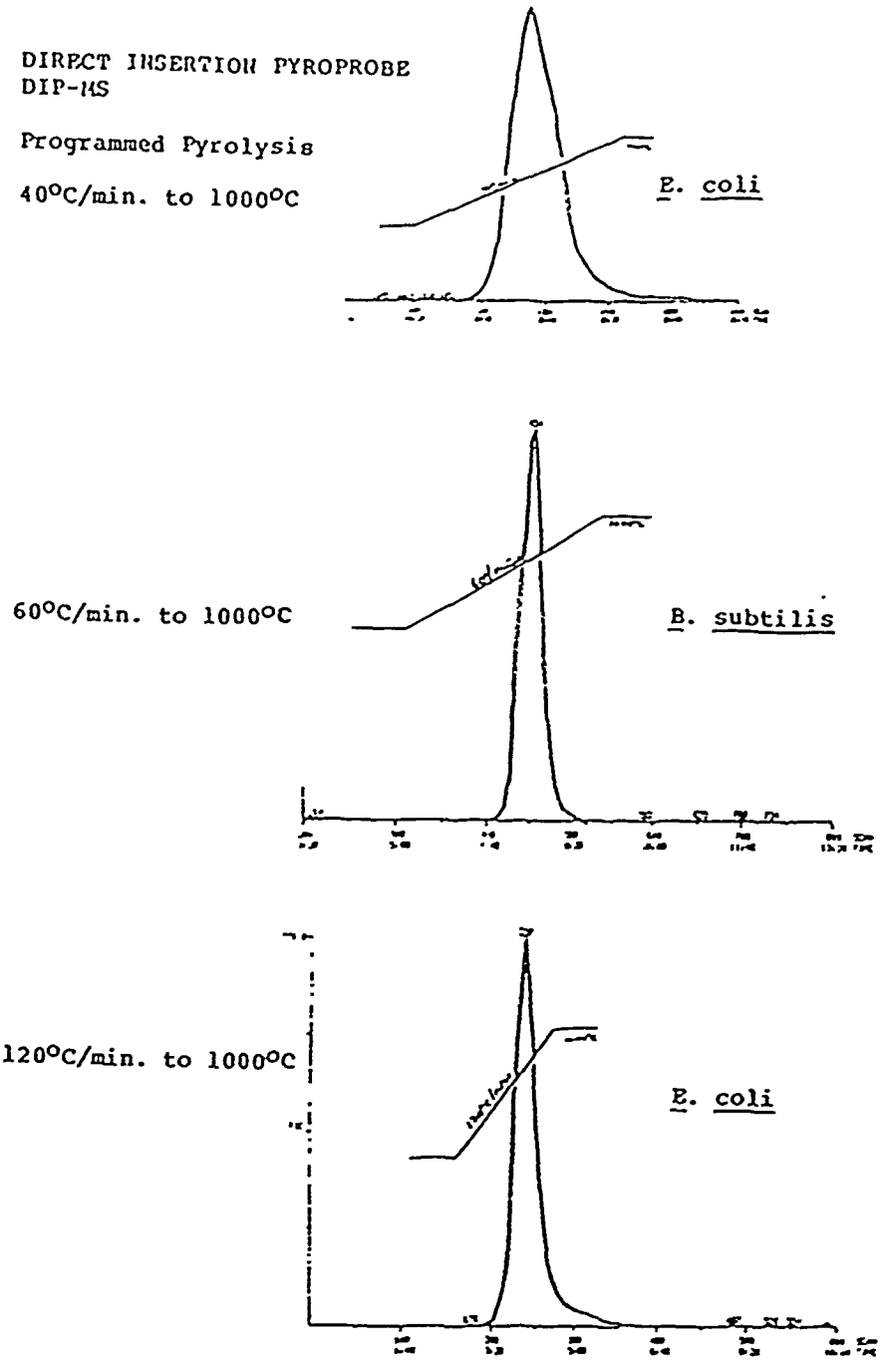


Figure 2. Direct Insertion Pyroprobe, DIP-MS, Programmed Pyrolysis

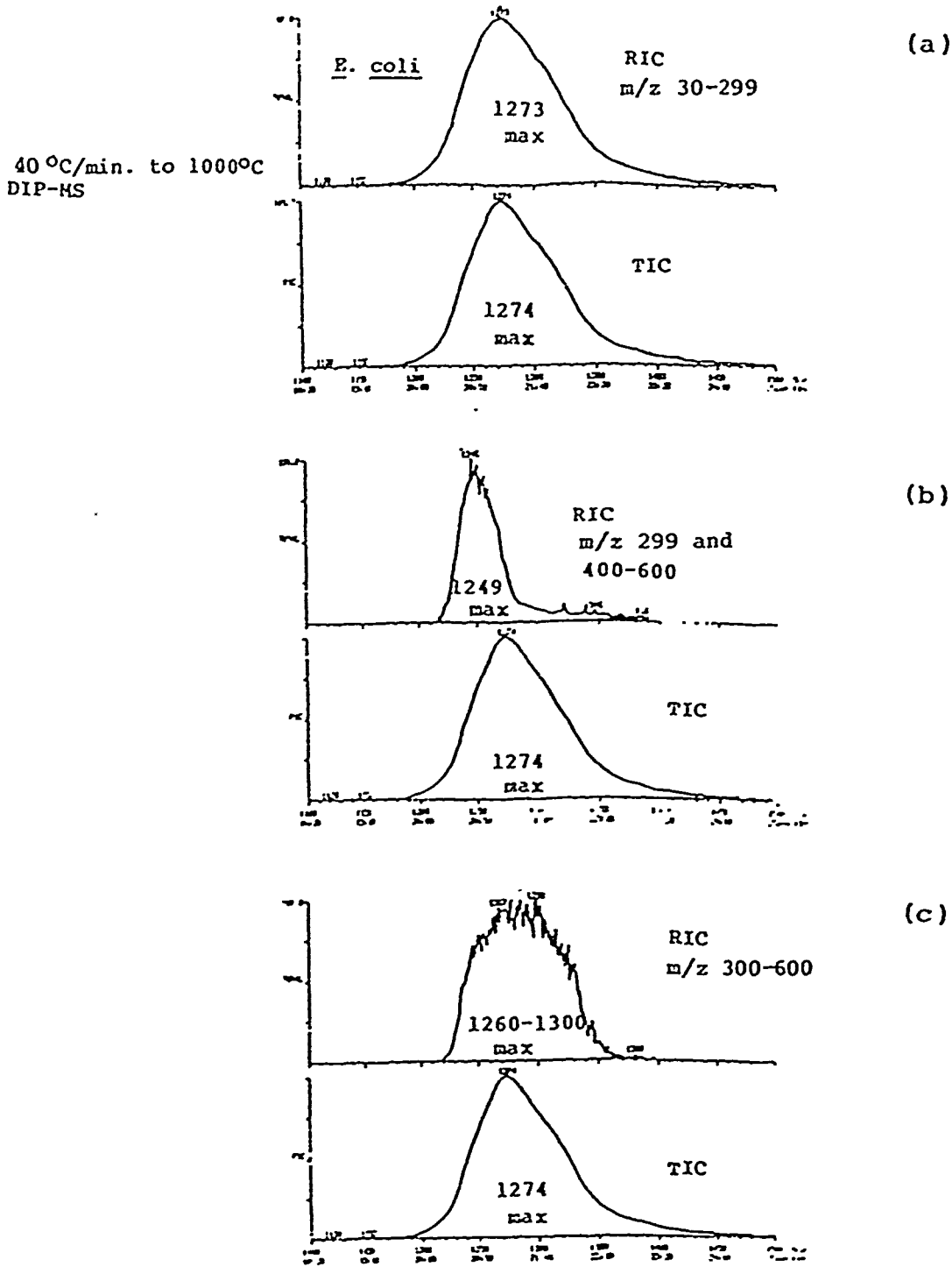


Figure 3. DIP-MS, E. coli, 40°C/min. to 1000°C

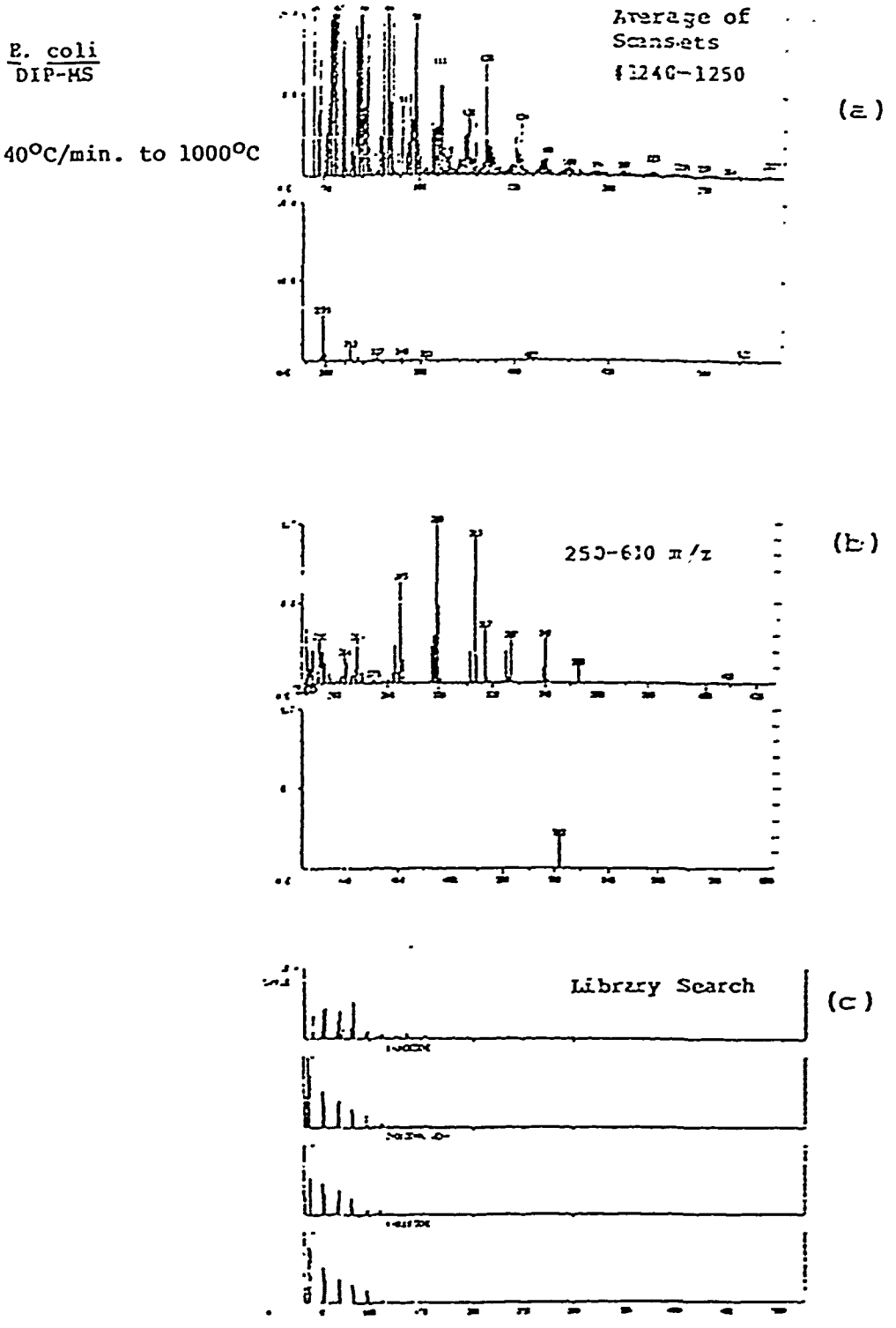


Figure 4(a-c). DIP-MS, E. coli, 40°C/min. to 1000°C

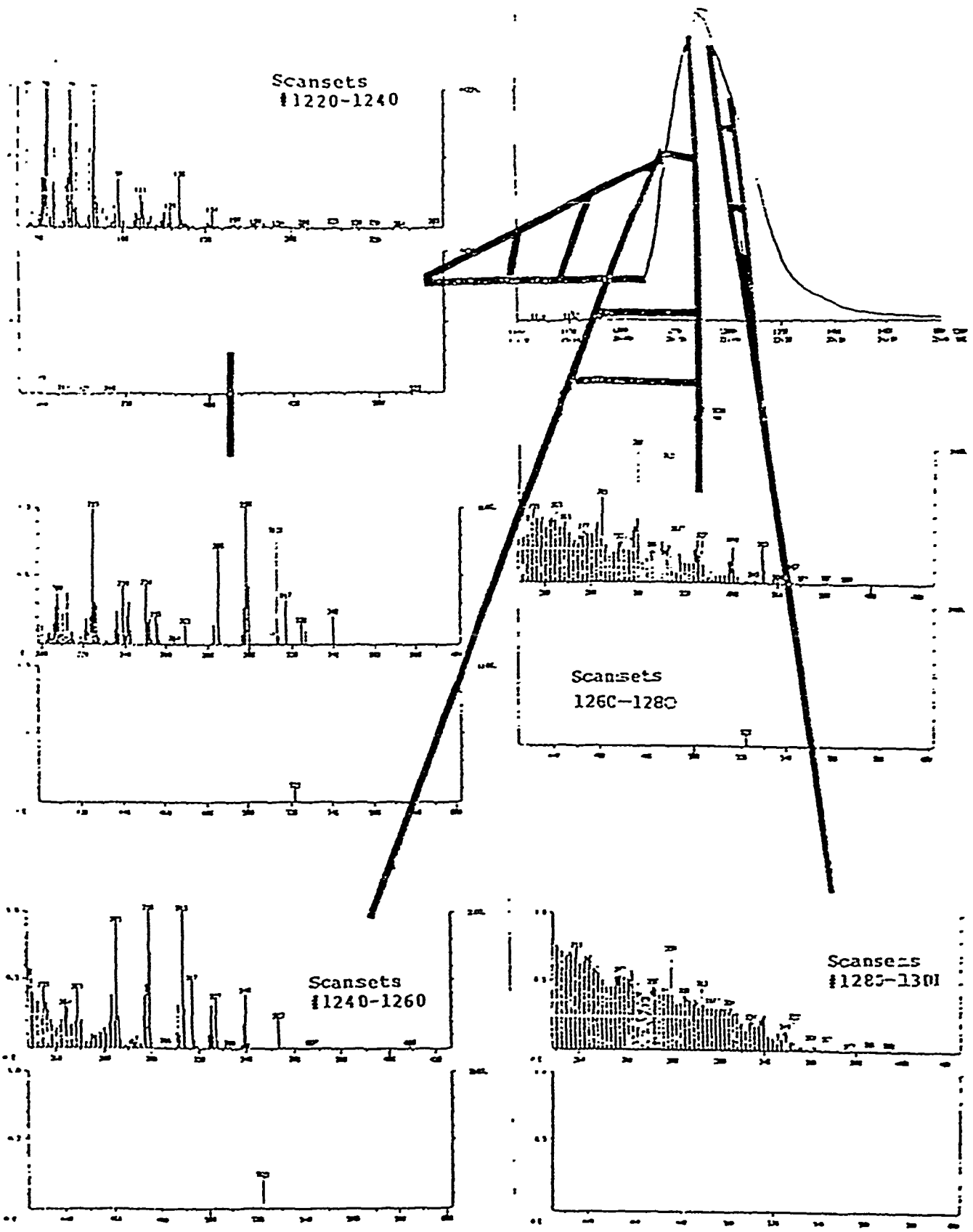


Figure 4(d). E. coli, 40°C/min. to 1000°C

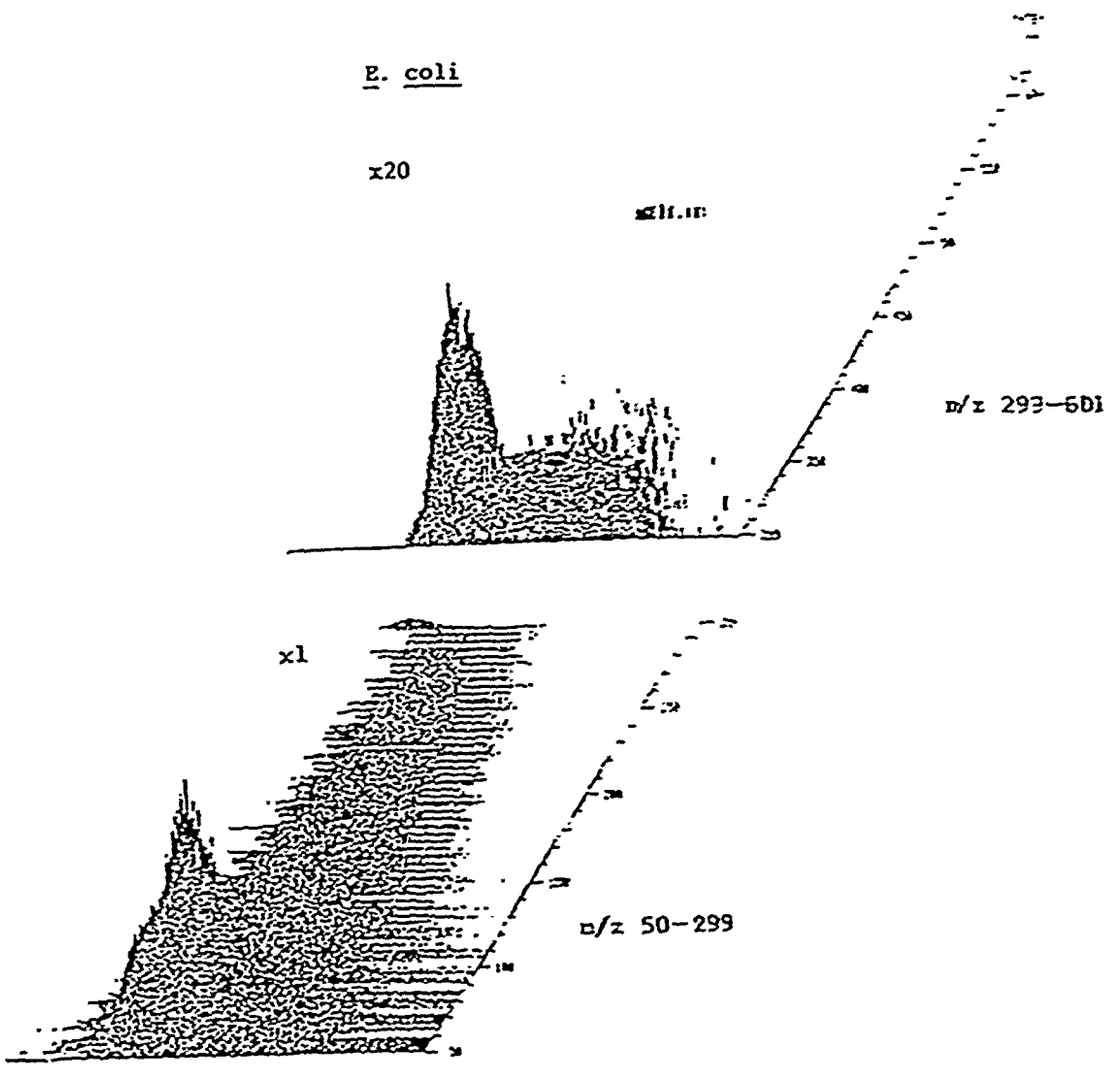


Figure 5. Pyrolysis Mass Map, DIP-MS, *E. coli*, 40°C/min. to 1000°C

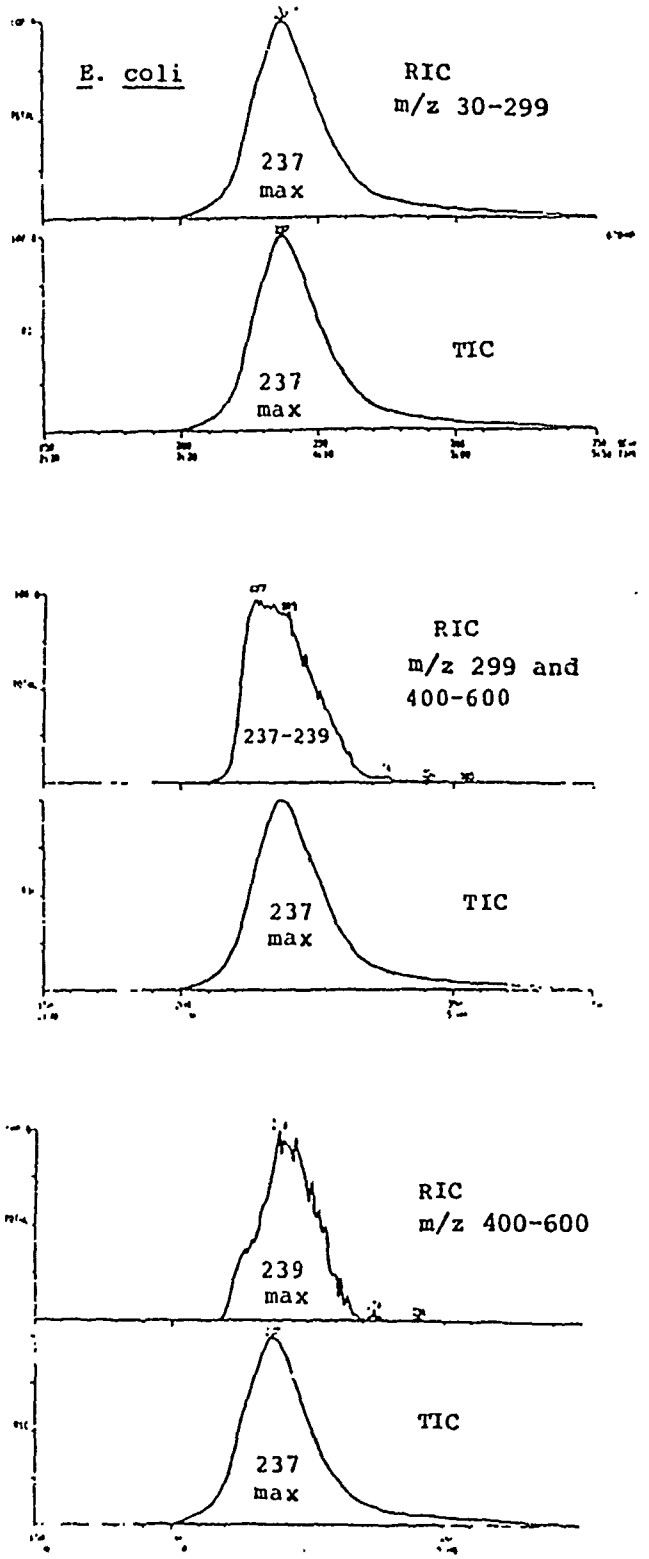


Figure 6. DIP-MS, E. coli, 120°C/min. to 1000°C

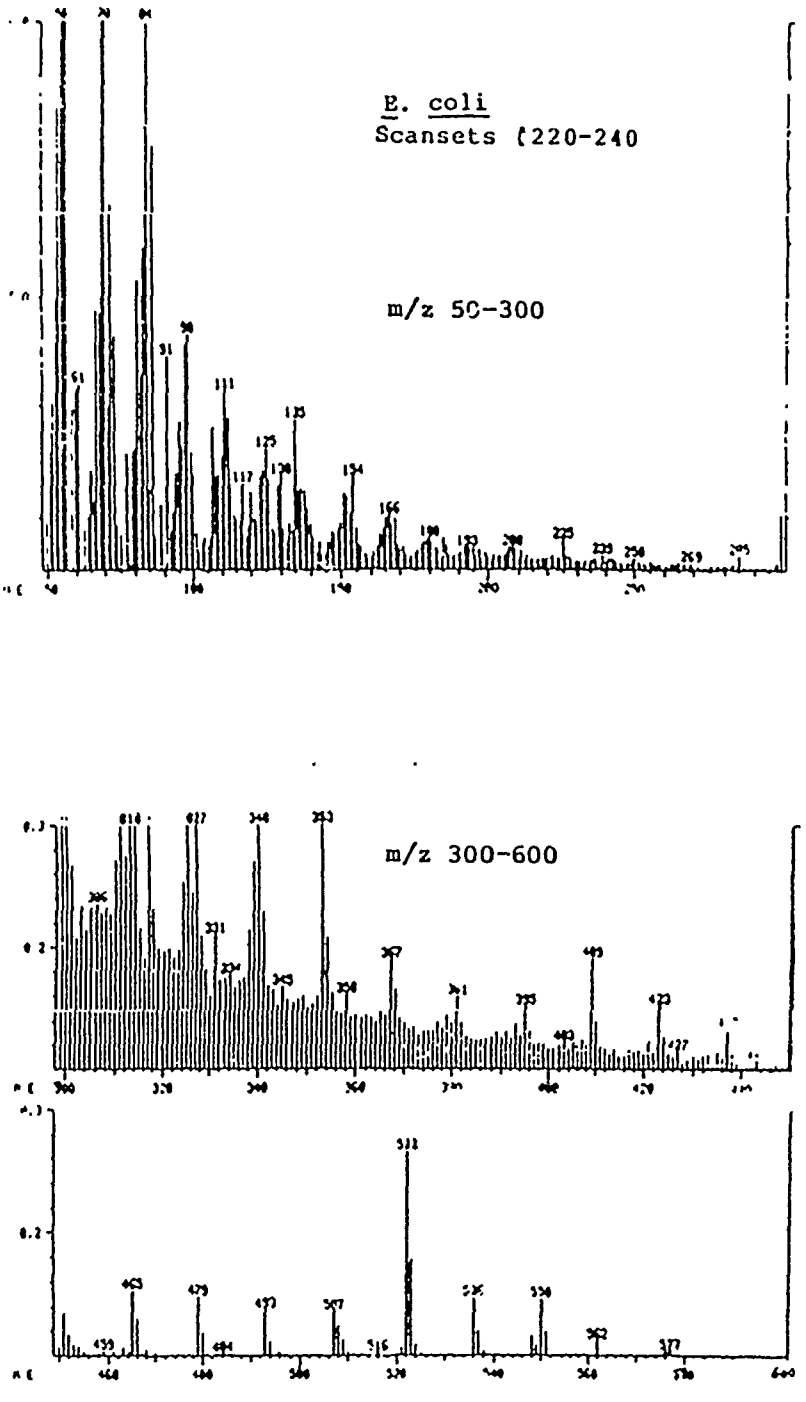


Figure 7(a-b). DIP-MS, E. coli, 120°C/min. to 1000°C

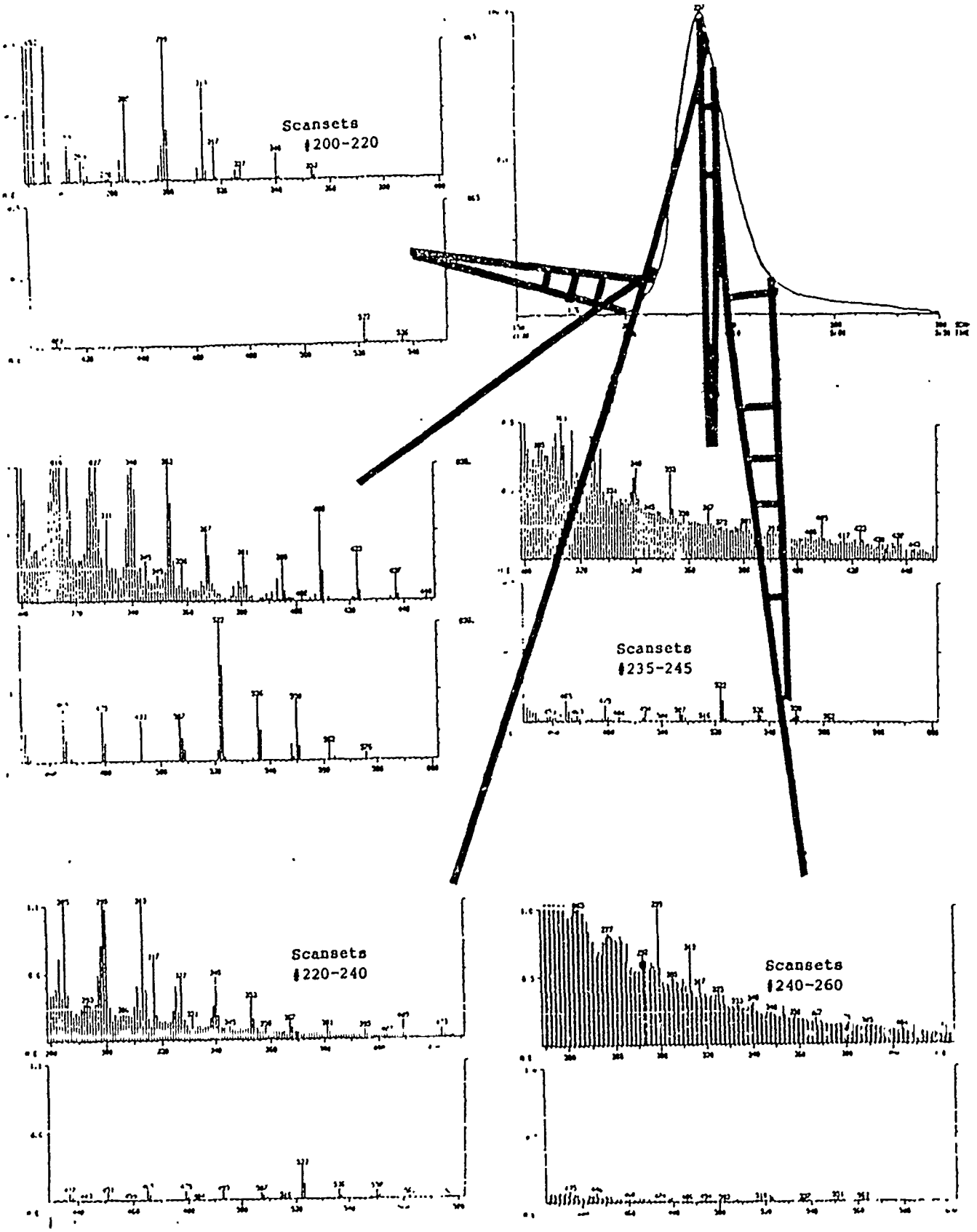
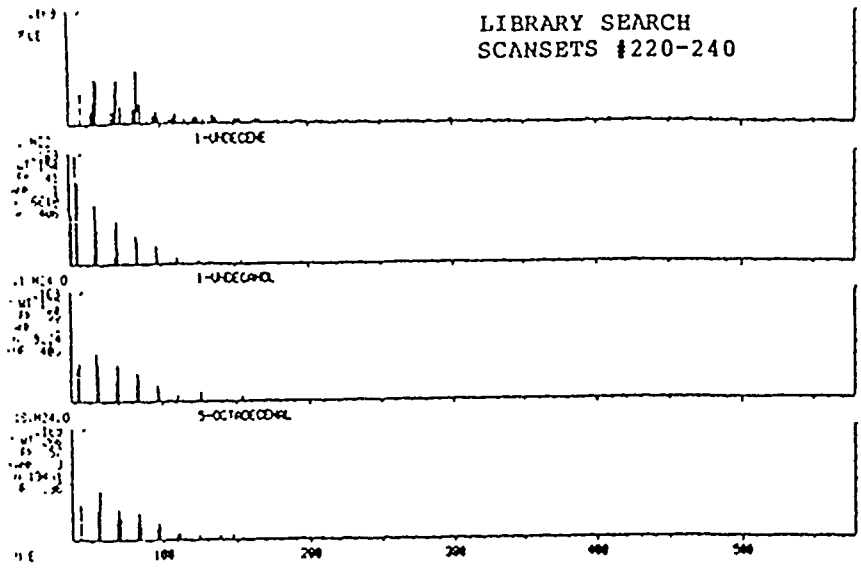


Figure 7(c). E. coli, 120°C/min. to 1000°C



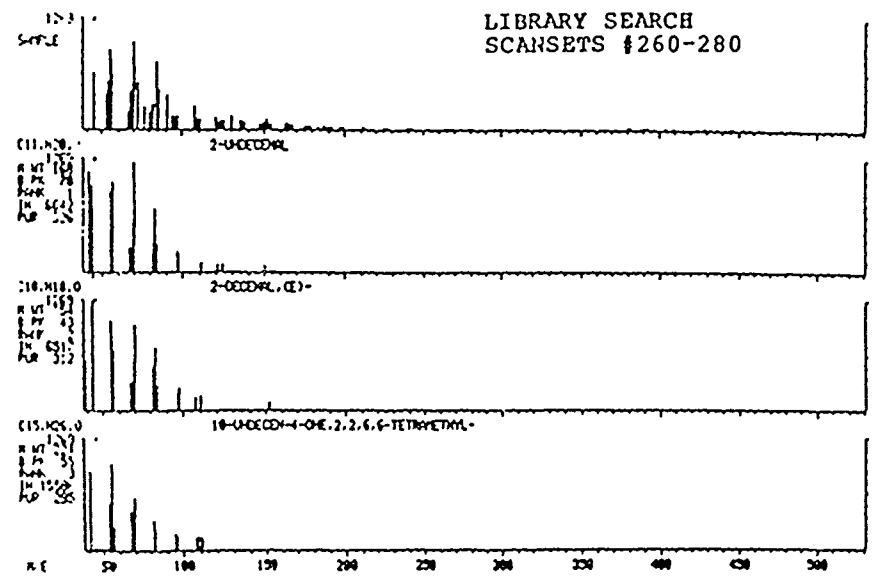


Figure 8. Library Search, Scansets #220-240, #260-280

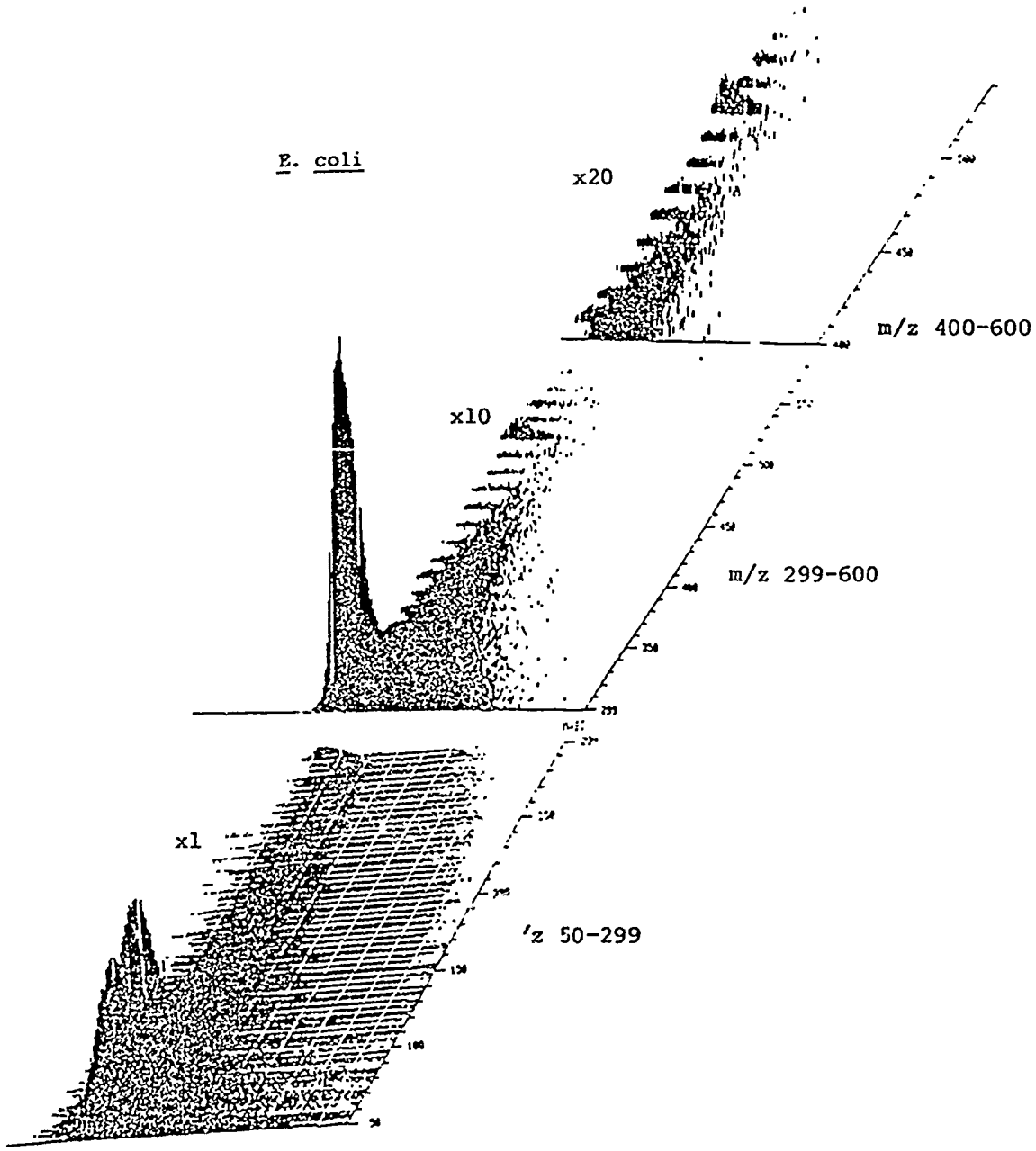


Figure 9. Pyrolysis Mass Map, DIP-MS, E. coli, 120°C/min. to 1000°C

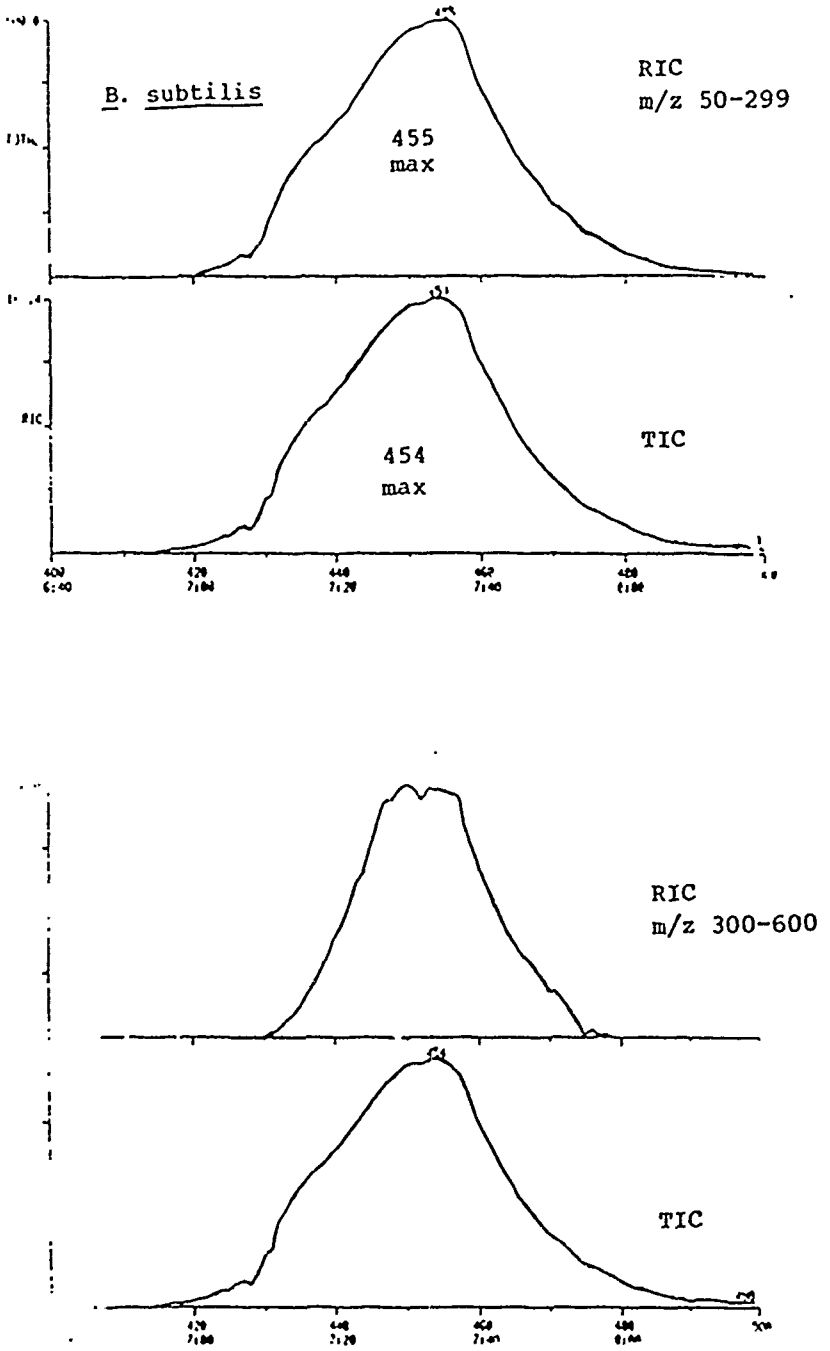
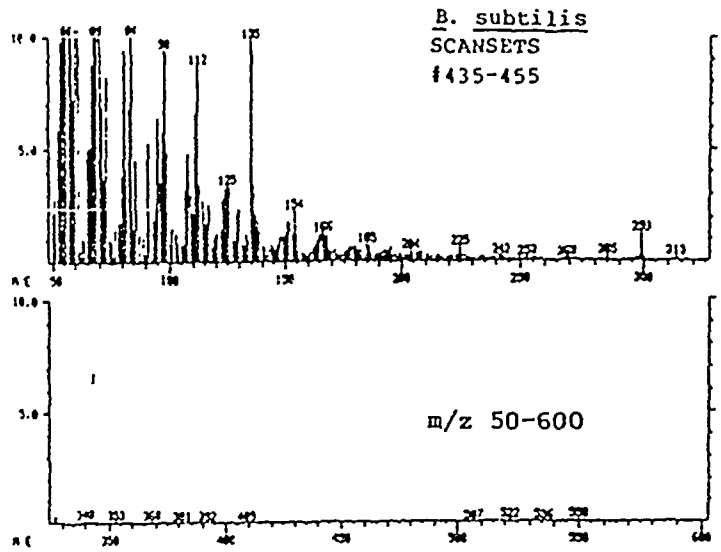
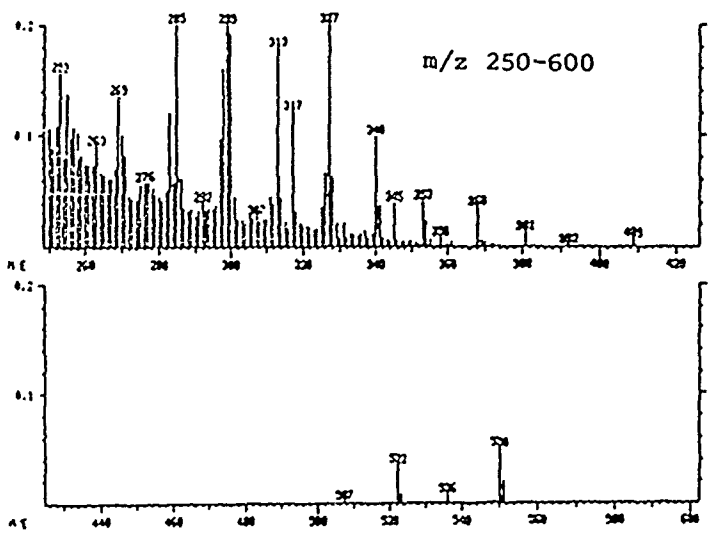


Figure 10. DIP-MS, B. subtilis, 60°C/min. to 1000°C



(a)



(b)

Figure 11(a-b). DIP-MS, B. subtilis, 60°C/min. to 1000°C

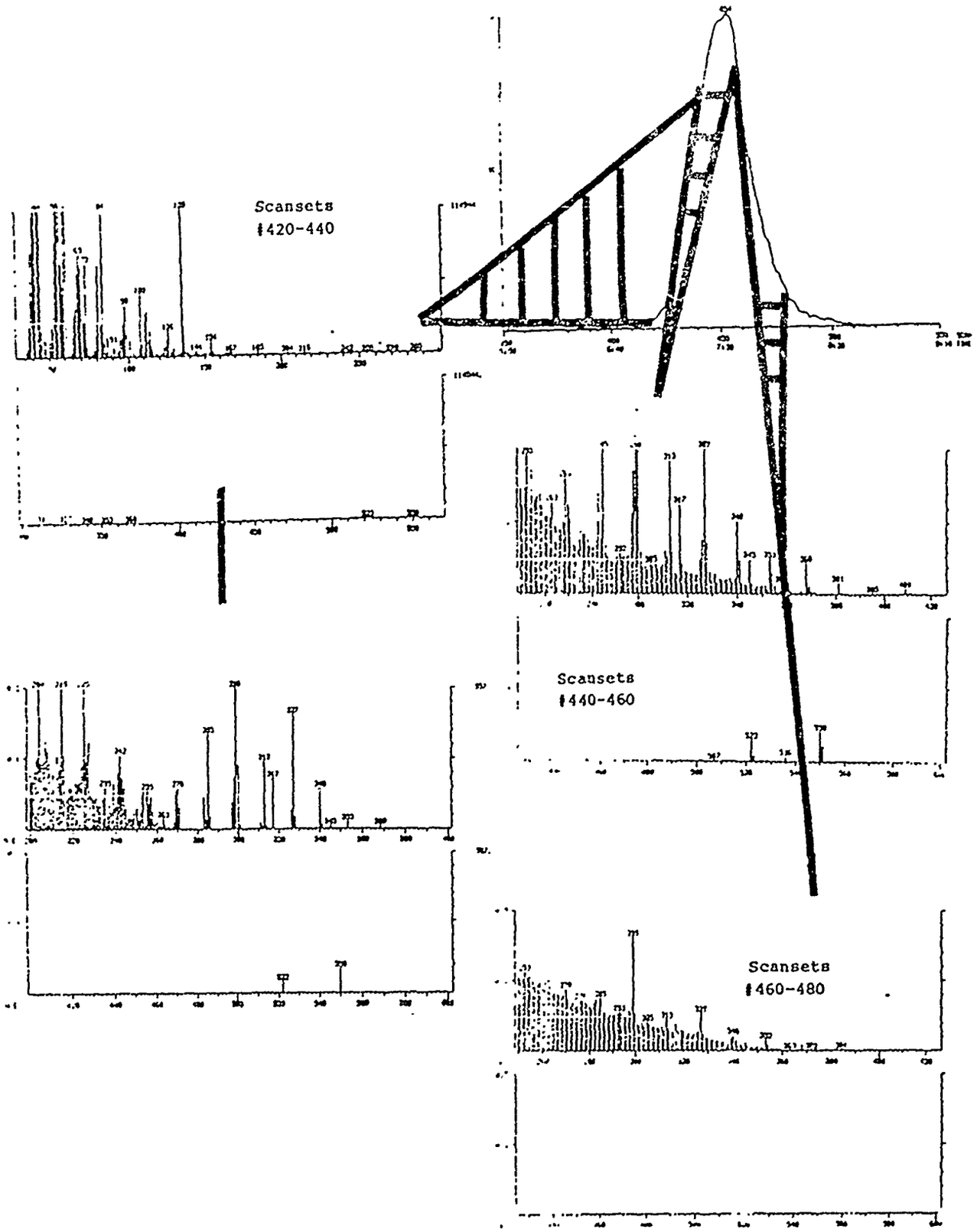


Figure 11(c). DIP-MS, B. subtilis, 60°C/min. to 1000°C

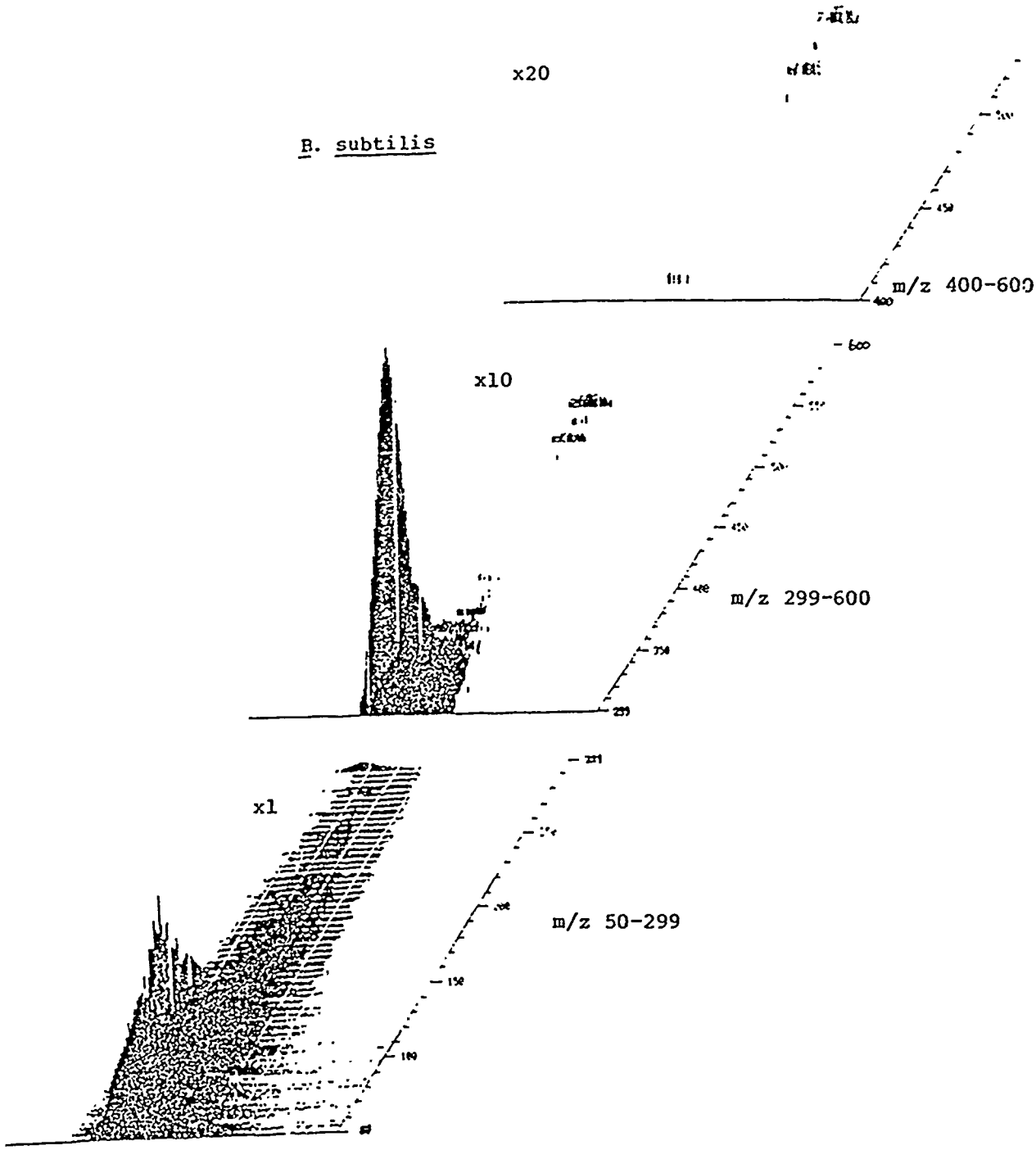


Figure 12. Pyrolysis Mass Map, DIP-MS, B. subtilis, 60°C/min. to 1000°C

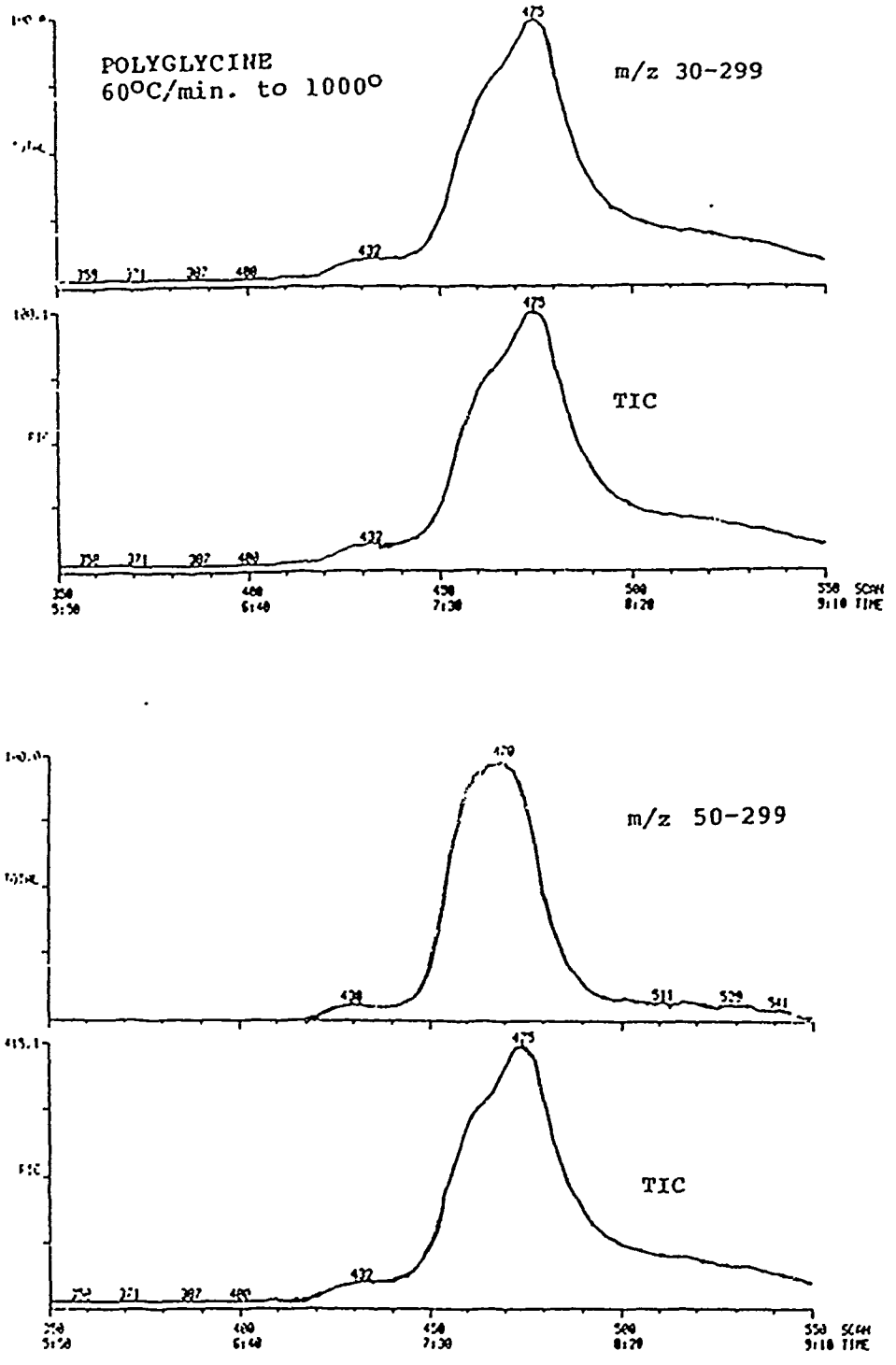
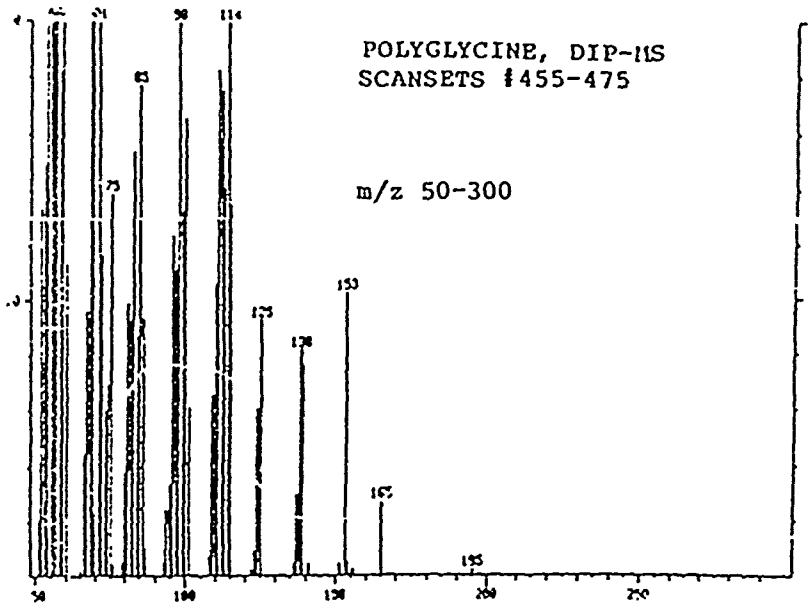


Figure 13. Polyglycine, DIP-MS, 60°C/min. to 1000°C



(b)

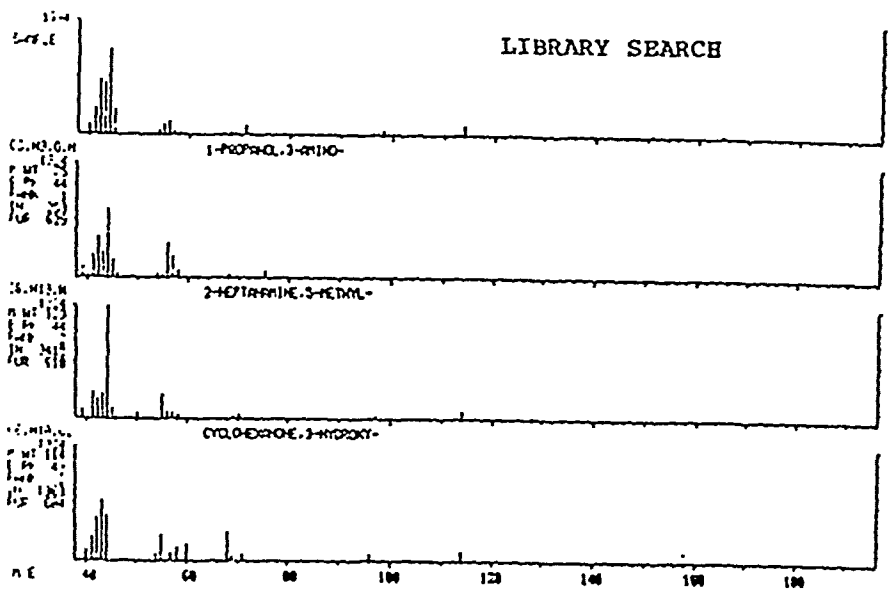


Figure 14. Polyglycine, DIP-MS, Scansets #455-475 and Library Search

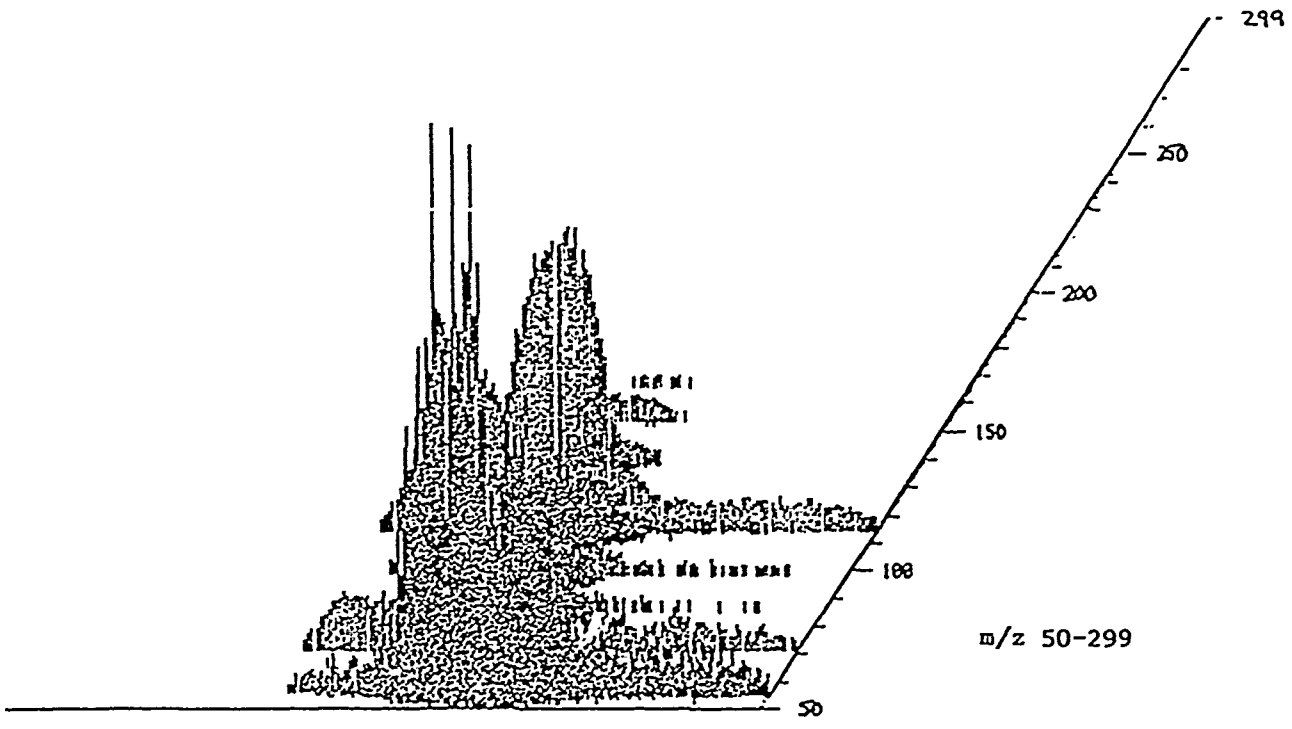
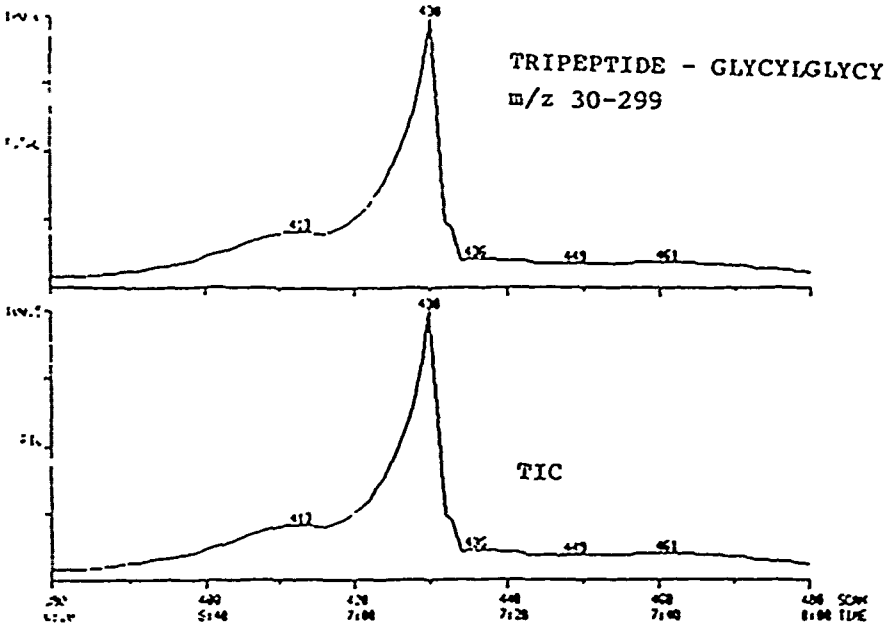


Figure 15. Pyrolysis Mass Map, Polyglycine, 60°C/min. to 1000°C

(a)



(b)

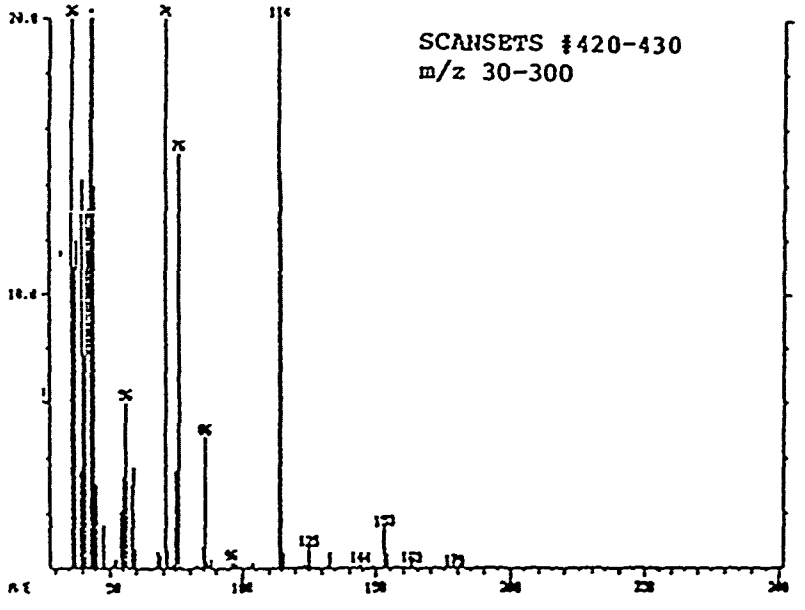


Figure 16. Tripeptide - Glycylglycylglycine and Scansets #420-430 60°C/min. to 1000°C

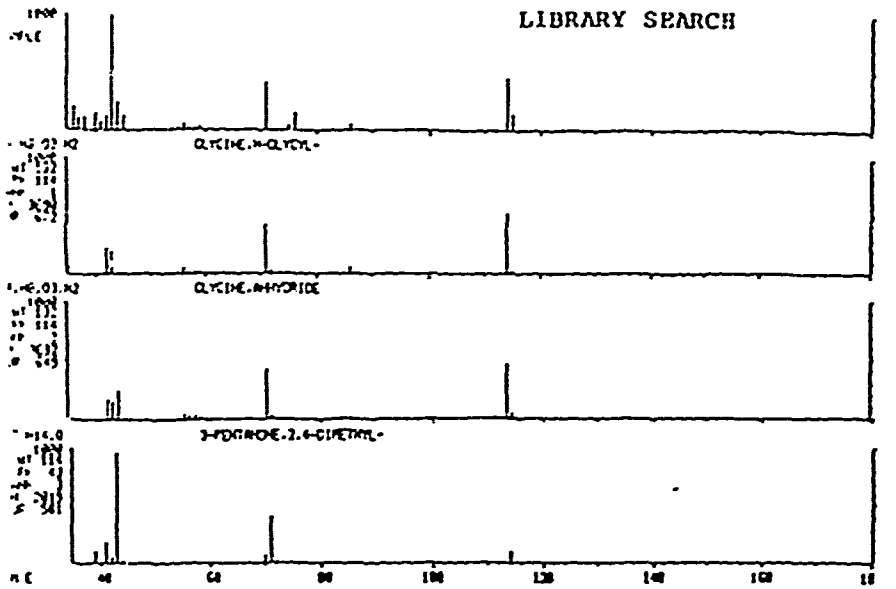


Figure 17. Library Search

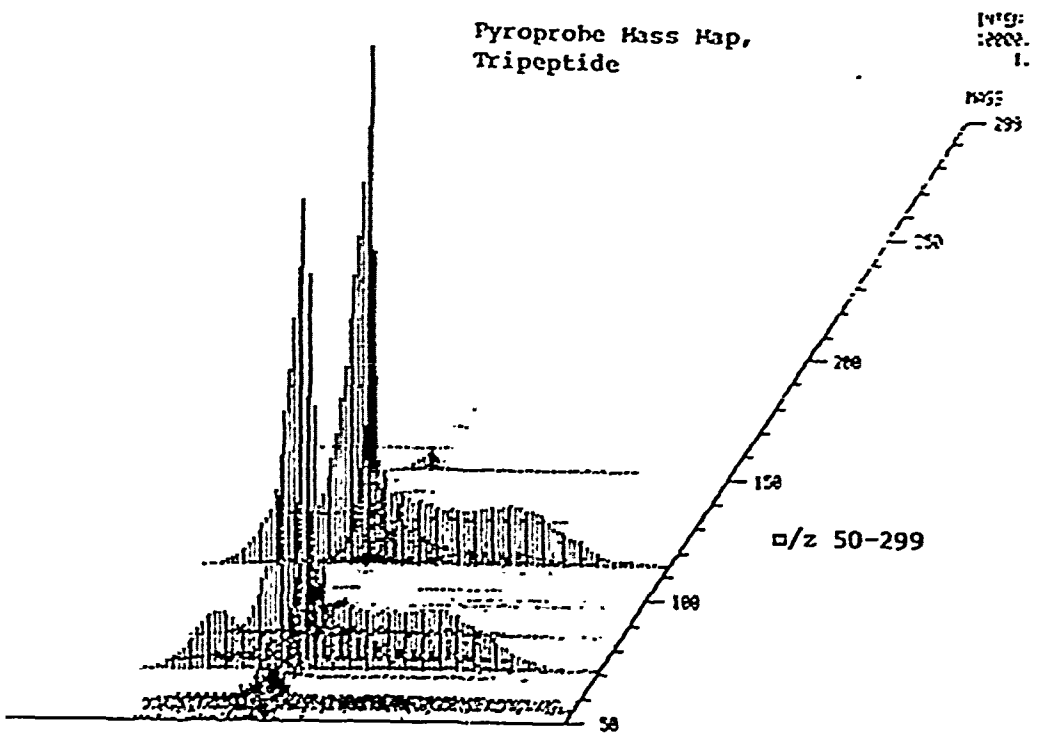
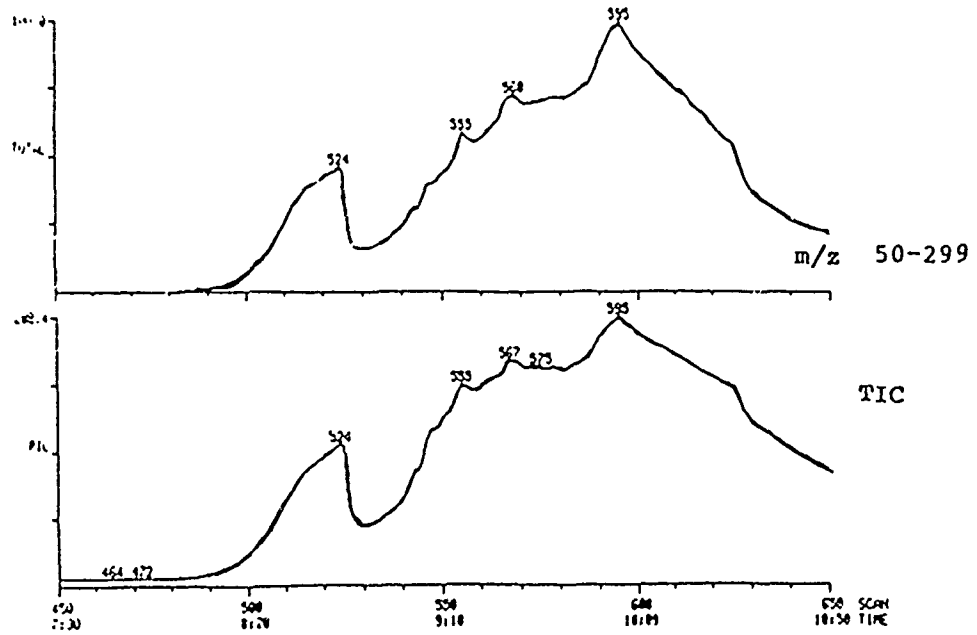
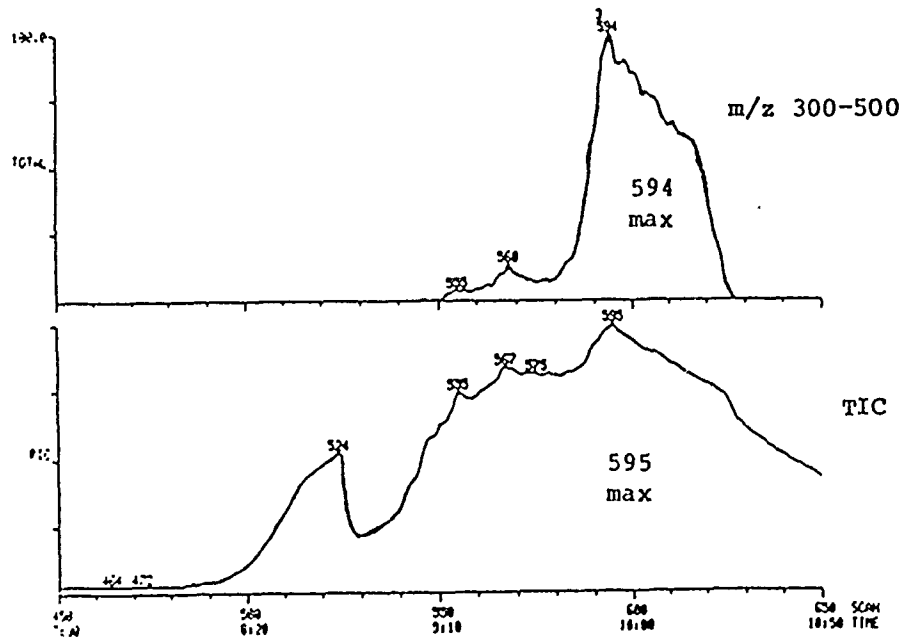


Figure 18. Pyrolysis Mass Map, Tripeptide, 60°C/min. to 1000°C



(a)



(b)

Figure 19. Murchinson Meteorite, Water Soluble, DIP-MS, 60°C/min. to 1000°C, MW 01, 9/26/90

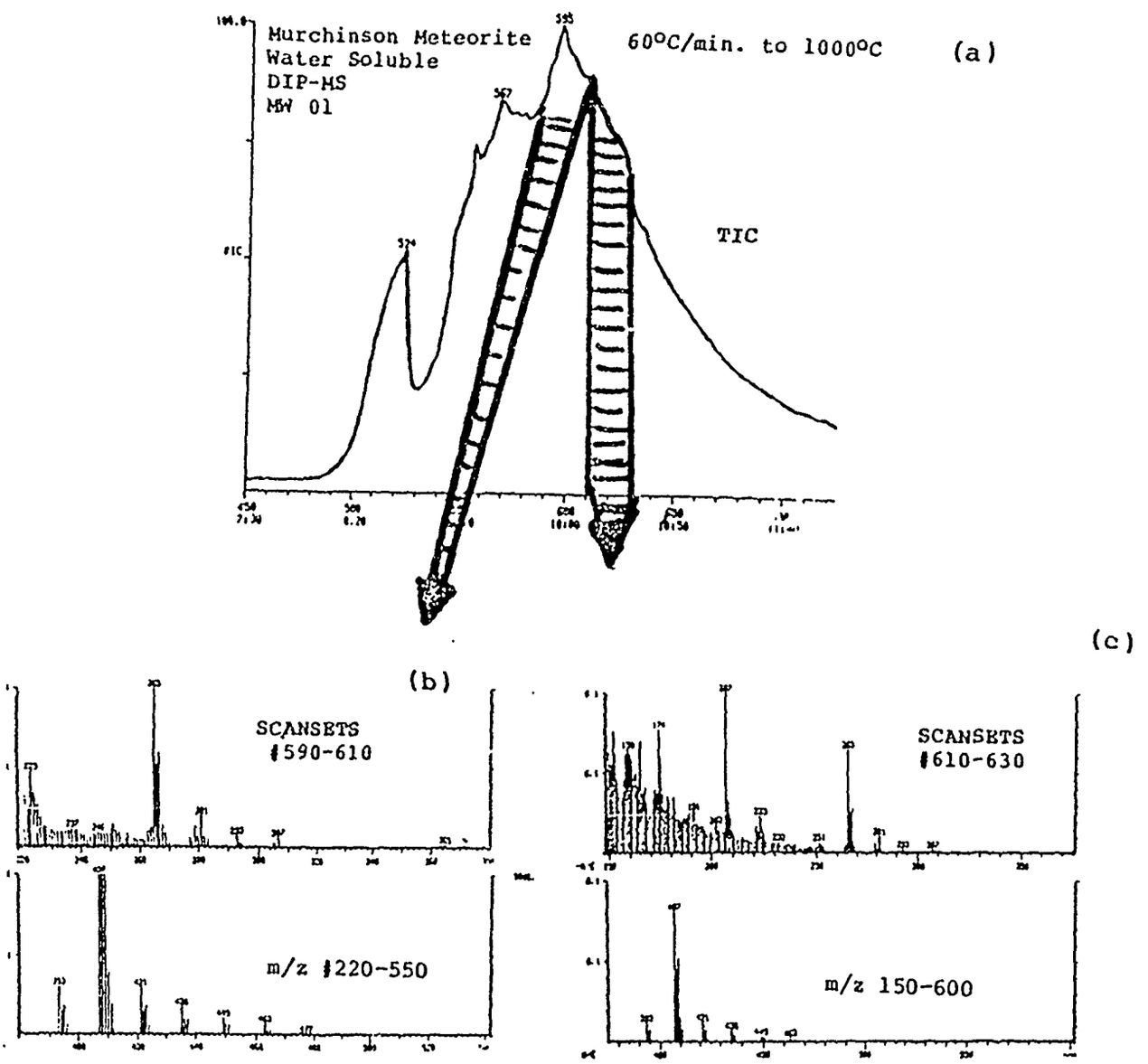


Figure 20. Murchinson Meteorite, Water Soluble, DIP-MS, 60°C/min. to 1000°C, MW 01

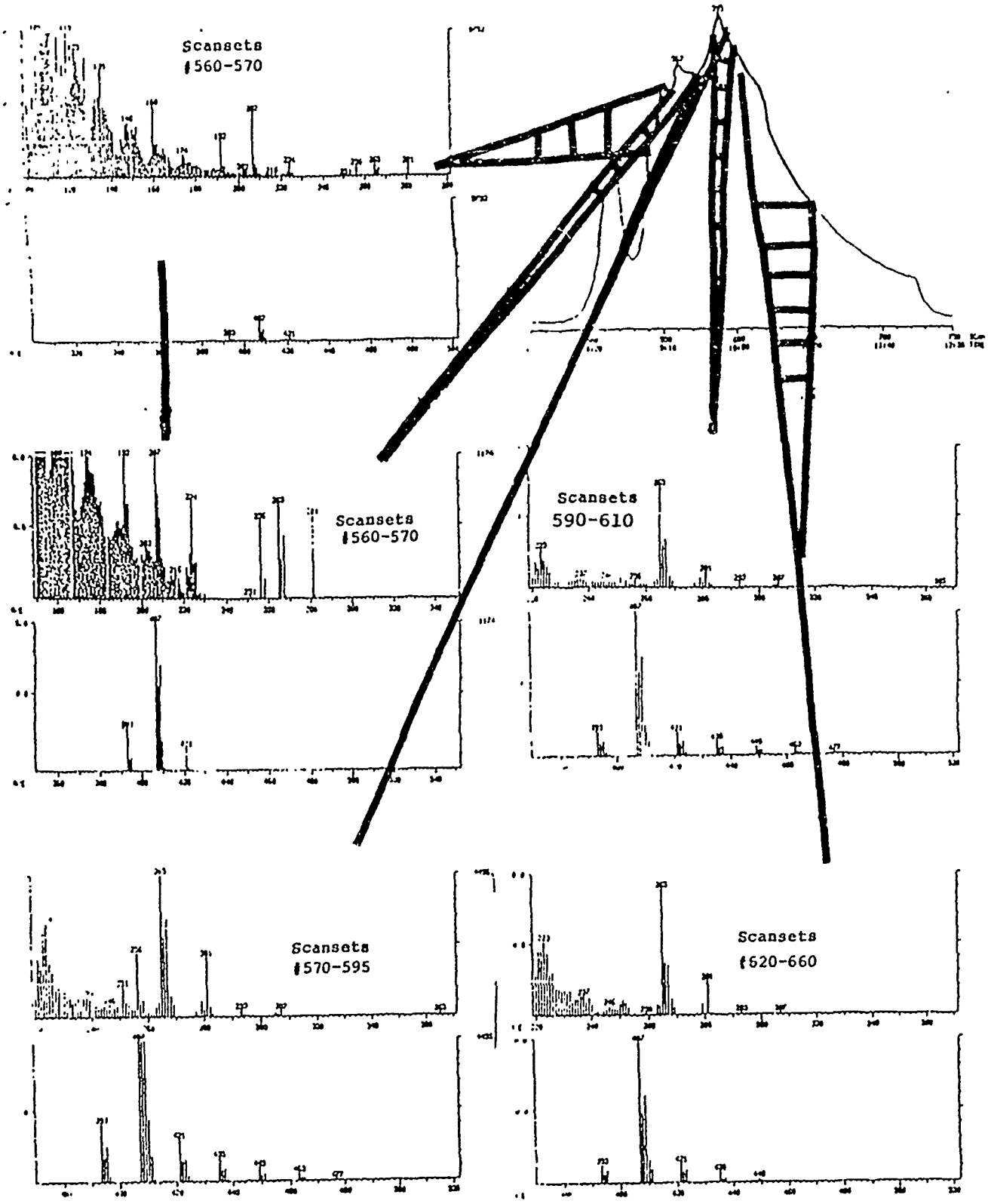


Figure 21. Meteorite Water Soluble, 60°C/min. to 1000°C

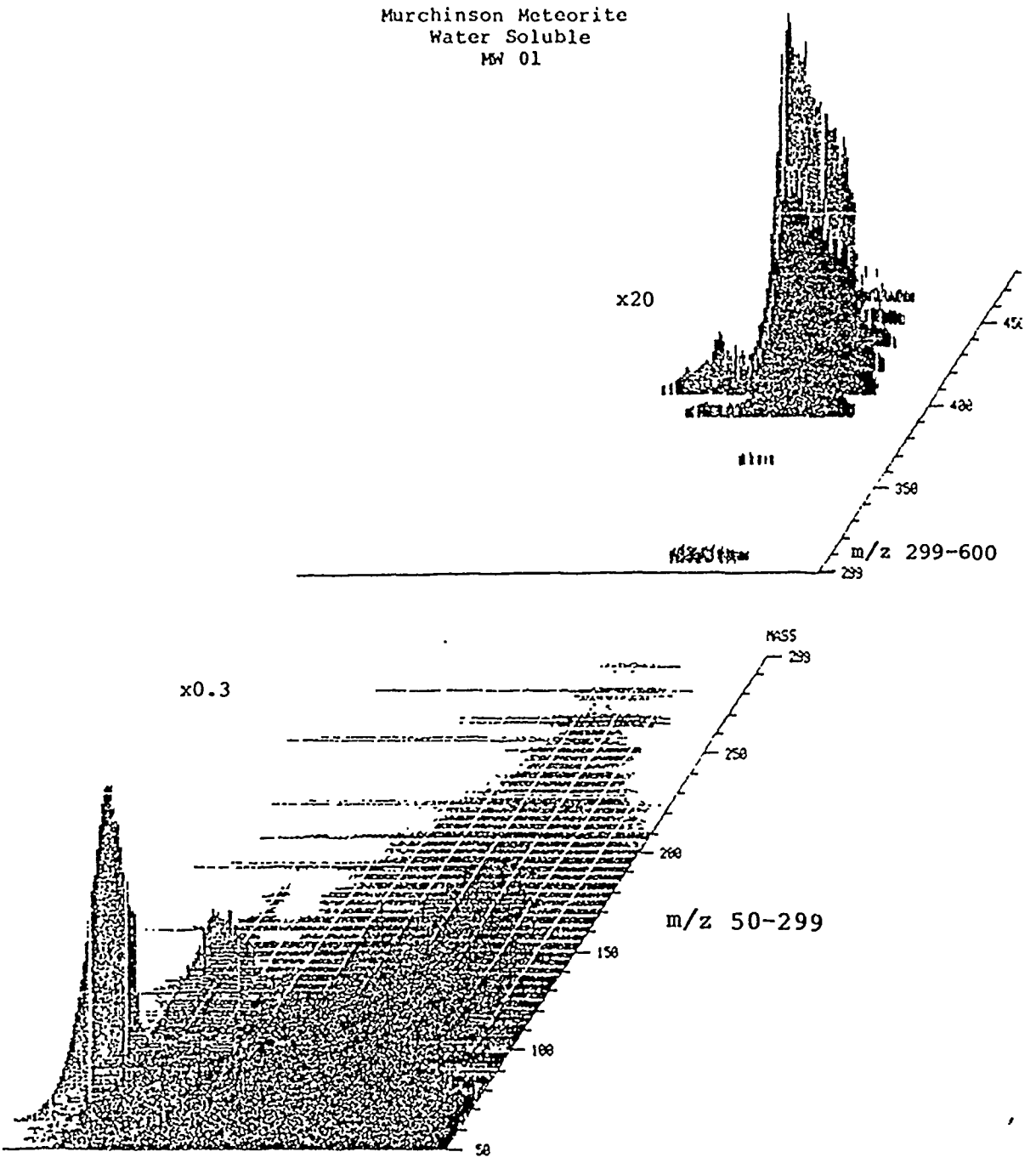


Figure 22. Pyrolysis Mass Maps, Murchinson Meteorite, Water Soluble, MW 01